

Effect of *Nigella sativa* Extract on Inflammatory Cells, Interleukin-10, Interferon- γ and Histological of Kidney in Monosodium Glutamate-Induced Rats

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ABSTRACT

Overconsumption of food additives monosodium glutamate (MSG), a flavour enhancer was unhealthy. Herbal medicine *Nigella sativa* (NS) has antioxidant properties able to cure the toxic induced by MSG. This study aimed to evaluate the risks of excessive use of MSG and to study the role of NS to inhibit inflammation and renal damage. Treated rats (twenty four male wistar rats) were divided into six group and analyzed by measuring the cells in blood, interleukin-10, interferon- γ serum levels by ELISA method and remove kidneys for histological examination. Histological of kidney for all groups except control, were showed different abnormalities include congestion of some blood vessels, hemorrhage between tubules, widening in the renal tubules, revealed severe dilatation of Bowman's capsule, shrinkage of glomeruli, and huge vacuole area, were observed compared with control. Interleukin-10 was reduced in Groups 2,3,4 and 5, whereas increase in group 6 compared with control. Interferon- γ was increased in groups 2,3,4 and reduced in groups 5,6 compared with control. Eosinophil was increased in groups 2,5 and reduced in groups 3,4 and 6. Basophil was reduced in groups 2, 3 and increased in groups 5 and 6 compared with control. This present study showed that administration of MSG to rats induced many changes effects on inflammatory cells, cytokines and histological of kidneys. NS has benefit in blood parameters, whereas harmful on kidney at these doses.

Keywords: eosinophil, interleukin-10, interferon- γ , kidney, monosodium glutamate, *Nigella sativa*

INTRODUCTION

Monosodium glutamate (MSG, $C_5H_8NO_4Na$, E621) is a type of salt which is used widely in most countries of the world, MSG one of the additives foods and one of the components of commercially processed foods. MSG added in foods because they can improve the food palatability and flavour enhancer. MSG has a special flavour that does not provide any vitamins or other enhancers [1]. MSG improves the taste of food which is called "umami" taste in Japan [2,3].

Monosodium glutamate is the sodium salt of glutamic acid, also known as the fifth taste. The basic sensory function of MSG is attributed to its ability to enhance the presence of other taste-active compounds.

Recommended for patients with missing desire or appetite for food also used in homes and restaurants [4] especially in Asian cuisine [5]. It is added to the food either as a purified monosodium salt or sodium salt as a component of a mixture of amino acids and small peptides resulting from the acid or enzymatic hydrolysis of proteins [6]. MSG are present in most canned prepared snacks and fast food. Glutamic acid is an amino acid naturally present in vegetables and foods of animal origin, such as cheese, and seafoods [4, 5, 7, 8, 9, 10, 11].

Over consumption of MSG for a long time led to many of the toxic effects, various inflammatory disease, positively related to obesity, and referred to the Chinese restaurant syndrome (CRS) [4, 8, 12]. Neuronal cell death is associated with glutamic production of free radicals in various organs. The brain is the most susceptible to oxidation due to a high rate of metabolic activity of oxidative stress [6, 7, 8, 10]. MSG causes kidney dysfunction, renal oxidative stress [13], and

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histopathological alterations in the kidney tissues of rats included revealed severe dilatation of Bowman's capsule, shrinkage of glomeruli, loss of brush border to see the relationship between expression of TLR4 and SRNS produced correlation value of 0.512 and its importance p value was 0.013 ($p < 0.05$). There is a difference significant expression of TLR4 on SRNS compared to SSNS with its importance value of 0.012 ($p < 0.05$) and there is also a significant correlation between expression of TLR4 and SRNS with its significance level of 0.013 ($p < 0.05$).

MATERIALS AND METHODS

Chemicals

Monosodium glutamate was obtained from local market in Malang, Indonesia and was dissolved in distilled water and given orally by gavage to rats. Black seeds (*Nigella sativa*) were obtained from local market in Malang, Indonesia. Black seeds will crush by mill mixer braun and its extract was prepare as suspension using distilled water and given orally in different doses by gavage to groups of treated male Wistar rats.

Animals

Healthy male wistar rats (2-3 months old littermate, 210 ± 30 g) were obtained from the Physiological Laboratory of Brawijaya University, Malang, Indonesia. Animals were placed on Animal Research Care. Adaptation time was done in one week to prepare the rats, that was weighing, examining the health status, and help the rats to adapt with new environment. Animals were housed in polypropylene cages and maintained at $25 \pm 2^\circ\text{C}$ and constant wetness degree according to the alteration of day and night.

Experimental design

Twenty four wistar rats were selected and randomly divided into six groups (four rats each). Group 1 served as control and was orally administered with distilled water throughout the experimental protocol. While Group 2 was orally administered 1 g of MSG at oral dose of 1 g/kg bw in distilled water for 30 days. Groups 3, 4, and 5 were administered 1 g of MSG per rat orally in the morning and NS (100, 200, and 400 mg) in the evening, respectively, for 30 days. Group 6 was orally administered 200 mg/kg bw of NS in distilled water for 30 days. Rats were sacrificed at the end of 30 days to take blood for measured the white blood cells differentiation count % using Sysmex XT-4000i Automated Hematology Analyzer device. Interleukin-10, interferon- γ levels in serum were measured by

ELISA Sandwich method. The kidneys were carefully dissected and removed. The tissues were fixed in 10% formalin, embedded in paraffin and that tissue sections (5 μm) were stained with hematoxylin-eosin to study their micro architecture by light microscopy.

Ethical issue

The study was approved by Health Research Ethics Committee (Medical Faculty, Brawijaya University) number No.237/EC/KEPK-S2/03/2015.

Statistical analysis

The results were expressed as mean \pm SD. Differences in the various parameters between groups were evaluated by one factor, One-way ANOVA test. All mean differences were considered significant if $p < 0.05$. Analysis were conducted using SPSS for Windows® v.17.

RESULTS AND DISCUSSION

Histological examination of kidney tissue in group 1 as control rats stained with Hematoxylin and Eosin stains (H&E) were normal kidneys with well demarcated cortex, medulla, normal Bowman's capsule and glomerulus as well as normal sized renal tubules as shown in (Figure 1; photos 1, 2). While histological examination of the kidney tissue after addition of MSG in group 2 rats shows different abnormalities (Figure1, photos 3, 4, 5). The abnormalities of kidney sections were summarized as congestion of some blood vessels, hemorrhage between tubules (Figure 1, photo 5), widening in the renal tubules (Figure1, photo 3), revealed severe dilatation of Bowman's capsule and shrinkage of glomeruli, and/or widening of the Bowman's space due to contraction of the renal glomerulus and hyper-cellularity (Figure1; photos 3, 4). In addition to huge vacuole area were also observed (Figure1, photo 4). The histological examination of kidneys tissue of groups 3, 4, 5, and 6, showed almost similar in their changed of structure of group 2, but had a lesser degree of these changes in group 3 compared with group 2, group 4 compared with 3, and groups 5,6 compared with 4, as followed figures: group 3 (Figure 1; photos 6, 7, 8), group 4 (Figure1, photos 9,10, 11), group 5 (Figure1; photos 12, 13, 14), and group 6 (Figure 1; photos 15, 16, 17).

Figure 2 shows that the average of interleukin-10 for each group is different each other significantly. The lowest average is in group 3 of 95.70 ± 23.06 , and the highest average is in group 6 at 219.85 ± 40.99 . Overall average of interleukin-10 is obtained by 134.99 ± 55.77 .

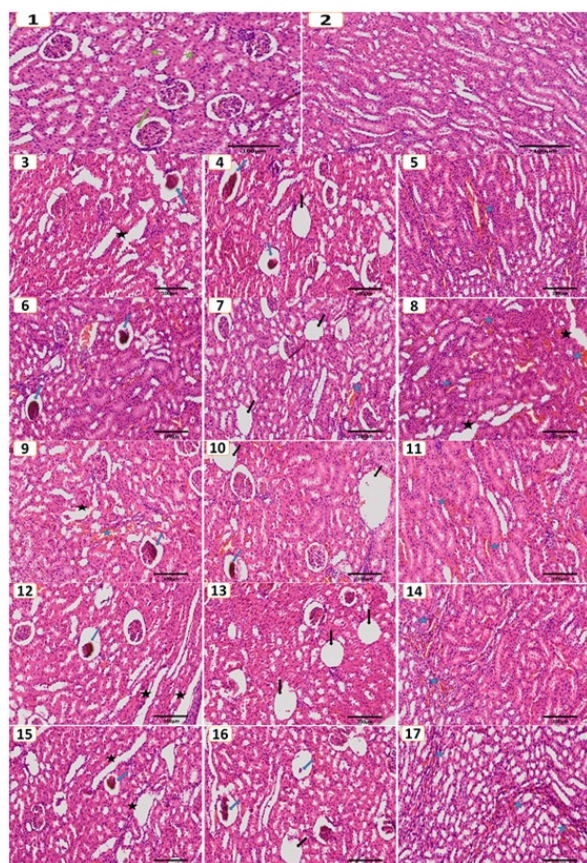


Figure 1. HE staining examination of histological kidney, Group 1 control group, Group 2 MSG group, Group 3 MSG & NS 100 mg/kg group, Group 4 MSG & NS 200 mg/kg group, Group 5 MSG & NS 400 mg/kg group, and Group 6 NS group (100× magnification). Green arrow normal Bowman's capsule and glomerulus. Green stars normal renal tubules. Blue stars congestion of some blood vessels, and hemorrhage between tubules. Black stars widening in the renal tubules. Blue arrow revealed severe dilatation of Bowman's capsule and shrinkage of glomeruli. Black arrow areas of huge vacuole.

Analysis of variance show a significance level of 0.00 is smaller than α (0.05), and the value of F count (15.12) is greater than F table 5% (2.77). These results indicate that there are differences of average that are significant (real) in the treatment of interleukin-10 factors.

Figure 2 shows also the average interleukin- γ each group was look different from each other significantly. The lowest average in group 5 of 264.000 ± 62.844 , and the highest average in group 2 at 771.500 ± 329.769 . Overall average of interleukin- γ 407.500 ± 239.527 . Analysis of variance showed a significance level of 0.014 is smaller than α (0.05), and the value of F count (3.932) is greater than F table 5% (2.773). From these

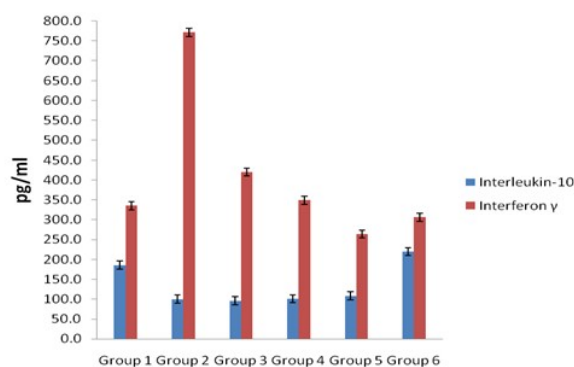


Figure 2. Data of interleukin-10 and interleukin- γ levels in serum rats for each group

results indicate that there are differences of average that are significant (real) in the treatment of interleukin- γ factors.

Table 1 shows that the average of each group is not too much different, as the following:

In eosinophil the lowest average is in group 3 of 2.88 ± 2.41 , and the highest average is in group 2 at 4.48 ± 2.58 . Overall average of eosinophil obtained by 3.28 ± 1.65 . Analysis of variance showed a significance level of 0.80 is greater than α (0.05), and the value of F count (0.46) is smaller than F table 5% (2.77). From these results indicate that there are differences of average that are not apparent in the groups of eosinophil factors.

In basophil the lowest average is in group 2 of 0.15 ± 0.10 , and the highest average is in group 6 at 0.48 ± 0.39 . Overall average of basophil obtained by 0.31 ± 0.21 . Analysis of variance showed a significance level of 0.37 is greater than α (0.05), and the value of F count (1.15) is smaller than F table 5% (2.77). From these results indicate that there are differences of average that are not apparent in the groups of basophil factors.

In neutrophil the lowest average is in group 1 of 25.03 ± 10.62 , and the highest average is in group 5 at 31.85 ± 8.69 . Overall average of neutrophil obtained by 28.24 ± 6.88 . Analysis of variance showed a significance level of 0.76 is greater than α (0.05), and the value of F count (0.52) is smaller than F table 5% (2.77). From these results indicate that there are differences of average that are not apparent in the groups of neutrophil factors.

In lymphocyte the lowest average is in group 5 of 59.83 ± 8.74 , and the highest average is in group 1 at 66.70 ± 8.01 . Overall average of lymphocyte obtained by 62.98 ± 6.83 . Analysis of variance showed a significance level of 0.753 is greater than α (0.05), and value

Table 1. Data of White Blood Cells Differentiation Count percentage in different groups

Group	Eosinophil		Basophil		Neutrophil		Lymphocyte		Monocyte	
	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD
1	3.1	1.32	0.33	0.15	25.03	10.62	66.7	8.01	4.85	1.79
2	4.48	2.57	0.15	0.1	29.4	5.65	60.93	7.99	5.05	1.09
3	2.88	2.41	0.25	0.13	25.78	4.04	65.18	4.15	5.93	2.19
4	3.08	1.26	0.33	0.17	29.9	2.84	61.88	5.25	5.13	1.65
5	3.2	1.22	0.35	0.13	31.85	8.69	59.83	8.74	6.2	1.73
6	2.98	1.09	0.48	0.39	27.5	8.5	63.38	7.93	5.68	1.43

of F count (0.53) is smaller than F table 5% (2.77). From these results indicate that there are differences of average that are not apparent in the groups of lymphocyte factors.

In monocyte the lowest average is in group 1 of 4.85 ± 1.79 , and the highest average in group 5 at 6.20 ± 1.73 . Overall average of monocyte obtained by 5.44 ± 1.58 . Analysis of variance showed a significance level of 0.833 is greater than α (0.05), and the value of F count (0.41) is smaller than F table 5% (2.77). From these results indicate that there are differences of average that are not apparent in the groups of monocyte factors.

MSG, a sodium salt of glutamic acid being added to Chinese food, canned vegetables, soups and processed food, acted as a flavour enhancement by stimulate the sensory receptors, thereby improving the palatability of food. Despite of its taste stimulation and appetite enhancement, various researchers had reported that it was toxic to humans and experimental animals [25]. Our results showed that histological examination of the kidney tissue after oral administration of MSG reveal different abnormalities. The abnormalities of kidney sections are summarized as congestion of some blood vessels, hemorrhage between tubules, widening in the renal tubules, reveal severe dilatation of Bowman's capsule and shrinkage of glomeruli. In addition, huge vacuola were also observed. This result was agreement with the study of Ortiz et al. and Mousa et al. [26, 27].

MSG has hepatotoxicity and nephrotoxicity tendencies especially when consumed at higher concentrations [28]. The effect of MSG addition to renal weretissue impairment, swelling of the lining epithelium of glomeruli, hydropic degeneration and vacuolization of the renal convoluted tubules, blood vessels dilatation and focal hemorrhage between the degenerative renal tubules, and many renal tubules of the rat kidneys

showed degenerative lesions under the effect of MSG. These are justifiable since the renal tubules are particularly sensitive to toxic influences due to their high oxygen consumption and vulnerable enzyme systems, complicated transport mechanisms that may be used for transport of toxin and maybe damaged by such toxin. The tubules also come in contact with toxic chemicals during their excretion and elimination by the kidneys [6]. One possible mechanism for the tubular lesions is the direct toxic effect on the cell function[14]. Alterations in the levels of lipid peroxides and antioxidants such as reduce glutathione, catalase and superoxide dismutase were observed in different organs and systems of adult rat during MSG treatment [6,29]. The effects observed in both liver and kidneys could have occurred because these organs are involved in the metabolism of glutamate [30]. MSG has a toxic effect on many body organs by altering ionic permeability of neural membrane and induced persistent depolarization. The mechanisms of MSG-induced damage include the production of free radicals that alter mitochondrial activity and genetic information [14].

Our results showed the toxic effect of *N. sativa*. Those are supported by Zaghlol et al. who reported that high doses of *N. sativa* oil had wide range of toxic effects on the kidney and liver. Some of these changes increase with doses additionof *N. sativa*. The previous study observed that the acute toxic effect of thymoquinone a main constituent of the *N. sativa* caused a significant increase in the concentration of serum creatinine and blood urea, which indicated serious effects on renal. High doses of *N. sativa* also caused cellular damage because of the alkylolation of proteins and DNA and formation of reactive oxygen species, including superoxide and hydrogen peroxide, which caused oxidative stress function [31]. These results suggest the possible toxicity of lethal doses of *N. sativa*. Whereas these

were different with previous study which report the protective effect of *N. sativa* against renal injury in rat kidneys [32].

In the present study, group 2 with only MSG treatment significantly decrease interleukin-10 compared with control group, this result was agreement with Hassan et al. IL-10 is an anti-inflammatory cytokine produced in large amounts from activated B-lymphocytes and play a regulatory role in suppressing harmful immune responses. IL-10 prevents the increase of nuclear factor kappa-light-chain-enhancer in activated B cells (NF- κ B) binding activity evoked by glutamate. There is a strong positive correlation among the changes of serum IL-10 levels and the loss of kidney anti-oxidants, this lends credence to the potential role that oxidative stress might be exit for mediating the immunotoxicity of MSG [4]. Our study showed that group 6 with only NS treatment more increase the interleukin-10 compared to control group, this result was agreement with [18, 20, 22, 23, 24]. In contrast, IL-10 has potential to be anti-inflammatory effects and suppress pro-inflammatory cytokines. The suggested that mechanism of *N. sativa* may affect both oxidative stress and inflammatory process simultaneously are through the inhibition of NF- κ B [20].

Our result showed that oral administration of MSG in group 2 are significantly increase the IFN- γ level compared with other groups. Earlier studies, which have also been confirmed this result on more recent reports on the adverse effects of MSG to increase levels of pro-inflammatory cytokines [33, 36]. The anti-inflammatory cytokines are series of immunoregulatory molecules that control the pro-inflammatory cytokine response [37]. MSG-treated animals develop obesity. In obesity, the intra-abdominal adipose tissue growth promotes increase pro-inflammatory cytokines infiltration and activation, which denotes the primary causes for chronic inflammation, morbidity and mortality risk [36].

In the present study, IFN- γ level in groups 3, 4 and 5 treated rats, were gradual decline respectively. Whenever the increasing dose of *N. sativa* would decrease the level of IFN- γ , and all of them were significantly more decrease compared to group 2 with only MSG treatment, it means that *N. sativa* may has strong ability to protect of MSG. In group 6 with only NS treatment showed that the IFN- γ level is also significant more decrease compared to group 2 MSG treatment. These results are similar to previous study that *N. sativa* has been proved experimentally to be an anti-inflammatory substance and it significantly reduced the

levels of pro-inflammatory mediators [20, 22, 23, 24]. *N. sativa* extract has been shown to have strong antioxidant properties to suppress the expression of inducible nitric oxide (NO) synthesis in rat macrophages. It has been shown that *N. sativa* extract has inhibitory effects on both the cyclooxygenase and the 5-lipoxygenase pathways of arachidonic acid metabolism and on membrane lipid peroxidation [38].

In the present study Group 2 was increased the eosinophil percentage compared with other groups. eosinophil possess a battery in their granules and lipid bodies that potent cytotoxic and pro-inflammatory agent [39]. Previous studies found that MSG associated with asthma [4, 8, 27, 29, 40] and autoimmune disease [41, 42] and these diseases may be the main causes to high percentage of eosinophil in blood. Groups 3, 4 and 5 were observed decrease compared with Group 2, it is mean NS has ability to protect of MSG and also we observed that at low dose of NS was more effect, and this result agreement with [43, 44]. previous studies revealed that an early influx of eosinophils into sites of inflammation precedes that of lymphocytes [45].

In the present study, the percentage of Basophil in Group 2 was decrease compared with other groups. We suggested that may basophils migrate from the circulation to the tissues. A decreased of basophil percentage known as basopenia may be due to severe allergy and Severe injury. Also recent genetic approaches indicate that basophils can migrate into lymphoid tissues and, in some circumstances, cooperate with other immune cells to promote optimal TH2 cytokine responses in vivo [46]. However it has been very difficult for most laboratories to obtain basophils without major contaminating cell populations, because the percentage of basophils in blood is low (< 1% of total WBC) and they share physicochemical properties with other blood cells. This lack of satisfactory purification protocols has considerably hampered basophil research and negatively affected the interests in this cell type. Basophils have also been proposed to play a key role in allergy by directly inducing the switch to the IgE isotope in B cells independently of T cells [47].

CONCLUSIONS

In conclusion, short term administration of MSG to rats in present study induced many changes effects on inflammatory cells. Cytokines and histological structure of the kidneys include congestion of some blood vessels. Hemorrhage between tubules. widening in the renal tubules. revealed severe dilatation of Bowman's capsule. shrinkage of glomeruli. and huge vacuole

area. This present study also demonstrated that *N. sativa* may improve inflammation and reduce oxidative stress in blood rats as well as increased anti-inflammatory cytokines as IL-10 and decreased pro-inflammatory cytokines as IFN- γ induced by MSG-treated rats. In addition, the result clearly indicate that overdoses of *N. sativa* may have toxic and harmful effect on the histological structure similar with MSG effect.

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